

# Colony PCR A, I and AI 0, 1 and 2

|                               | PCR 20 µL reaction (units : µL) | Final Concentration |   | Colony:  |       |           |              |
|-------------------------------|---------------------------------|---------------------|---|----------|-------|-----------|--------------|
| 10x thermopol reaction buffer | 2,5                             | 1X                  |   |          |       |           |              |
| 10 mM dNTPs                   | 0,5                             | 200 µM              |   |          |       |           |              |
| 10 µM Forward Primer          | 0,5                             | 0.2 µM              |   |          |       |           |              |
| 10 µM Reverse Primer          | 0,5                             | 0.2 µM              |   | Primers: |       |           |              |
| Template (colony)             | 0                               | 5 ng                |   |          |       |           |              |
| DMSO (optional)               | 0                               | 3 %                 |   |          |       |           |              |
| Taq polymerase                | 0,125                           | 1.0 units/50 µl PCR |   |          |       |           |              |
| Nuclease-free Water           | 20,875                          |                     |   |          |       |           |              |
| Total volume                  | 25                              |                     |   |          |       |           |              |
|                               |                                 |                     |   |          |       |           |              |
|                               |                                 |                     |   |          |       |           |              |
| Number of Reactions           | 28                              |                     | Colonies 1, 2 and 3 of each gRNA and negative control |          |       |           |              |
|                               |                                 |                     |   |          |       |           |              |
| 10X Reaction buffer           | 70                              |                     | A0-1  | I0-1     | AI0-1 | 1-2-3 neg |              |
| dNTPs                         | 14                              |                     | A0-2  | I0-2     | AI0-2 |           |              |
| f_Rmv_DsRed2HH                | 14                              |                     | A0-3  | I0-3     | AI0-3 |           |              |
| r_IDT_dia                     | 14                              |                     | A1-1  | I1-1     | AI1-1 |           |              |
| Taq Polymerase                | 3,5                             |                     | A1-2  | I1-2     | AI1-2 |           |              |
| Water                         | 584,5                           |                     | A1-3  | I1-3     | AI1-3 |           |              |
| Total volume                  | 700                             |                     | A2-1  | I2-1     | AI2-1 |           | Prgm Y-COL-D |
| Volume per tube               | 25                              |                     | A2-2  | I2-2     | AI2-2 |           |              |
|                               |                                 |                     | A2-3  | I2-3     | AI2-3 |           |              |
|                               |                                 |                     |   |          |       |           |              |
|                               |                                 |                     |   |          |       |           |              |
| Number of reactions           | 19                              |                     | Colonies 4 and 5 of each gRNA and negative control    |          |       |           |              |
|                               |                                 |                     |   |          |       |           |              |
| 10X Reaction buffer           | 47,5                            |                     | A0-4  | I0-4     | AI0-4 | 4-5 neg   |              |
| dNTPs                         | 9,5                             |                     | A0-5  | I0-5     | AI0-5 |           |              |
| f_Gbs_DsRed2HH                | 9,5                             |                     | A1-4  | I1-4     | AI1-4 |           |              |
| r_Gbs_dia                     | 9,5                             |                     | A1-5  | I1-5     | AI1-5 |           |              |
| Taq Polymerase                | 2,375                           |                     | A2-4  | I2-4     | AI2-4 |           | Prgm Y-COL-E |

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|                         |  |          |                  |      |       |  |  |
|-------------------------|--|----------|------------------|------|-------|--|--|
| Water                   | 396,625  |          | A2-5             | I2-5 | AI2-5 |  |  |
| Total Volume            | 475  |          |                  |      |       |  |  |
| Volume per tube         | 25   |          |                  |      |       |  |  |
|                         |  |          |                  |      |       |  |  |
| Replating:              | Colonies 1, 2 and 3 of each gRNA combination (A0, A1, A2, I0, I1, I2, AI0, AI1, AI2) |          |                  |      |       |  |  |
|                         | 21 --> Nothing and AI0-3 is in number 22   |          |                  |      |       |  |  |
|                         |  |          |                  |      |       |  |  |
| Replating 2:            | Colonies 4 and 5 of each combination (numbers 1 to 18)                               |          |                  |      |       |  |  |
|                         | CYC3: 27 to 31   |          |                  |      |       |  |  |
|                         |  |          |                  |      |       |  |  |
| Programs:               |  |          |                  |      |       |  |  |
|                         |  |          |                  |      |       |  |  |
| PCR program A 'Y-COL-D' |  |          | Colonies 1, 2, 3 |      |       |  |  |
| Initial Denaturation    | 95°  | 7 min    |                  |      |       |  |  |
|                         | 95°  | 30 sec   |                  |      |       |  |  |
|                         | 52°  | 1 min    |                  |      |       |  |  |
| 35 Cycles               | 67°  | 1 min 20 |                  |      |       |  |  |
| Final Extension         | 72°  | 5 min    |                  |      |       |  |  |
| Hold                    | 4°   |          |                  |      |       |  |  |
|                         |  |          |                  |      |       |  |  |
| PCR program A 'Y-COL-E' |  |          | Colonies 4, 5    |      |       |  |  |
| Initial Denaturation    | 95°  | 7 min    |                  |      |       |  |  |
|                         | 95°  | 30 sec   |                  |      |       |  |  |
|                         | 52°  | 1 min    |                  |      |       |  |  |
| 2 Cycles                | 67°  | 1 min 20 |                  |      |       |  |  |
|                         | 95°  | 30 sec   |                  |      |       |  |  |
|                         | 65°  | 1 min    |                  |      |       |  |  |
| 30 Cycles               | 67°  | 1 min 20 |                  |      |       |  |  |
| Final Extension         | 72°  | 5 min    |                  |      |       |  |  |
| Hold                    | 4°   |          |                  |      |       |  |  |
|                         |  |          |                  |      |       |  |  |



## Colony PCR A, I and AI 0, 1 and 2

[illegible]